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Evaluation of Apparent Digestibility of Fish Meal, Sunflower Meal and Rice Polishings for *Labeo rohita*

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Abstract

The apparent digestibility of dry matter (DM), protein (CP), fat and gross energy (GE) of three feed ingredients Fish meal, sunflower meal and rice polishing for *Labeo rohita* was studied by using chromic oxide as a dietary marker. The experimental diets consisted of a mixture of reference diet 69.3 percent and test ingredients 29.7 percent. Apparent nutrient digestibility coefficient of DM was highest (67.77% ± 6.81) for fish meal followed by sunflower meal (38.18% + 0.63) and rice polishing (21.63% ± 20.46). Apparent CP digestibility was highest (88.84% ± 4.90) for the fish meal and was followed by sunflower meal (71.46% ± 1.54) and rice polishing (70.22% ± 8.62). For fat apparent digestibility was highest (82.37% ± 6.26) for sunflower meal and next highest values in the descending order were (80.36% ± 3.07) for fish meal and (38.38% ± 1.51) for rice polishing. The apparent digestibility for GE was highest (93.64% ± 9.34) for fish meal and this was followed by the rice polishing (77.47% ± 10.02) and sunflower meal (67.29 % ± 3.50).

Key words: Nutrient digestibility, *Labeo rohita*, Feed ingredients.

Introduction

Aquaculture is expanding as an industry throughout the world and the need for specialized feeds designed for particular production situations is increasing. The nutritionists and feed manufacturers have concentrated their efforts on determining which of the wide variety of feedstuffs available to the feed industry may be used to produce lower cost fish feed.

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Assessing the quality of feedstuffs is best done, by conducting extensive feeding trials but they are time-consuming and expensive. The next best method of quality assessment is to measure apparent digestibility coefficients using an *in-vivo* procedure. Digestibility is one of the most important aspect in evaluating the efficiency of feedstuffs. Digestibility co-efficient by themselves are limited expressions of nutritive value, but are common means of evaluating feedstuffs. The digestibility of nutrients contained in the feedstuff could be used to access the suitability of feedstuff as the ingredient in fish feed. Determining the digestibility of nutrients in a feedstuff is important not only to enable formulation of diets that maximize the growth of cultured fish by providing appropriate amounts of available nutrients, but also to limit the wastes produced by the fish.

The nutritional value of a feedstuff depends not only on its chemical composition but also on the digestibility of its nutrients and energy. Therefore, knowledge of the Apparent Digestibility Coefficients (ADC) of nutrients of feedstuffs is essential for correct diet formulation.

The various studies concluded that digestibility of nutrients and energy from various feedstuffs varies from fish to fish. Digestibility studies have been mainly conducted on other fish species like trout (Nose, 1960; Inaba *et al*., 1962; Kitamikado *et al*., 1964), channel cat fish (Smith and Lovell, 1971, 1973), common carp (Scherbina and Kazaluskene, 1971) and tilapia (Popma, 1982, Hanley, 1987), however, nutrient availability from conventional and non-conventional feedstuffs in major carps has not been studied (Singh, 2000). Present study designed to investigate nutrients digestibility of local available feed ingredients for major carps (*Labeo rahita).*

Materials and Methods

Experimental fish

Fingerlings *Labeo rohita* were obtained from the Government Fish Seed Hatchery, Satiana, Road, Faisalabad, Pakistan. The fingerlings were acclimatized for one week in cemented tanks (2.4 x 1.3 x 0.9m). During this period fish were fed once daily to apparent satiation on the reference diet used in subsequent digestibility study (Allan and Rowland,

1992). Before experiment, fish were treated with 5g/l NaCl to ensure fish were free from ecto-parasites and to prevent fungal infection (Rowland and Ingram, 1991).

Feed ingredients and diet preparation

All tested diets (Table 1) were composed of 69.3 percent reference diet and 29.7 percent test ingredients (fish meal, sunflower meal and rice polishings) on dry matter (DM) basis. Chromic oxide was used as an inert marker and incorporated into the reference diet and test diets at 1 percent inclusion level (Table 1).

Table 1: Ingredients percentage and chemical composition of reference and test diets (DM basis)

Ingredient	Reference diet	Test diet I	Test diet II	Test diet III
Rice polishing	7.0	4.9	4.9	4.9
Fish meal	12.0	8.4	8.4	8.4
Sunflower	10.0	7.0	7.0	7.0
Maize gluten 60%	10.0	7.0	7.0	7.0
Wheat bran	15.0	10.5	10.5	10.5
Soybean	28.0	19.6	19.6	19.6
Rice broken	5.0	3.5	3.5	3.5
Cod liver oil	10.0	7.0	7.0	7.0
Vitamin premix	1.0	0.7	0.7	0.7
Minerals	1.0	0.7	0.7	0.7
Chromic oxide	1.0	1.0	1.0	1.0
Fish meal	-	29.7	-	-
Rice polishings	-	-	29.7	-
Sunflower	-	-	-	29.7
Total	100.0	100.0	100.0	100.0

Reference and test ingredients were ground and sieved for incorporation into diets. All dry ingredients were mixed in mixer for 30 minutes, where after, fish oil was gradually added, while mixing constantly. Eighty-five ml (85 ml) of water per 100g of feed was slowly blended into the mixer, resulting in a suitably texture dough, as for fish food (Lovell, 1989). Drying was carried out in a convection oven at 35 ^{0}C for 48h. The dry product was cut into pellets of 2.5 mm diameter. The above procedure was followed to produce a reference and three test diets. Proximate chemical analysis of the experimental diets is shown in Table 2.

Experimental system

A four week experiment was conducted independently in glass aquaria, specially designed for the collection of faecal material, having two chambers, i.e. feeding compartment (about one third of the aquarium volume) and collecting compartment with sloping walls and a removable faecal collecting glass tray at the bottom.

Feeding protocol and faecal collection

After acclimatization, fingerlings were transferred into glass aquaria (90x46x38 cm) via random interspersion. For each treatment three replicates were used and in each replicate eight fingerlings were stocked (average weight, 19g). Fish were fed at the rate of 2 percent of live wet weight on their prescribed diet twice daily (morning and afternoon) in the feeding chamber. After a feeding session of 2 hours, fingerlings were shifted in the faecal collecting chamber. Twice daily, faecal samples were collected after removing the water from the collecting chamber. The faecal collecting glass tray was removed from the bottom of faecal collecting chamber and poured the faeces into labeled petri dishes and left uncovered in a refrigerator at 1-3 ^{0}C for overnight drying. Faeces were stored in a sealed bottle for analysis. Faecal collection continued for 30 days when it was judged that a sufficient sample had been collected for chemical analysis.

Analytical procedure

A representative sample of feed or dried faeces was homogenized using a motor and pestle and analyzed essentially by AOAC (1990) procedures: dry matter (DM) by oven drying at 105 ^{0}C for 16 h; crude protein (CP) by micro-kjeldahl analysis and gross-energy by oxygen bomb calorimetry. Total lipid was determined through 1045 soxtec system HTz and chromium estimation by using acid digestion method, (Furukawa and Tsukahara, 1966).

Apparent digestibility coefficients calculation

Apparent digestibility coefficient of nutrients for each diet and test ingredients was calculated according to Maynard and Loosli (1969) and Cho and Slinger (1979).

Statistical analysis

The data was statistically analyzed by using "Analysis of Variance" (ANOVA), 'Duncan's New Multiple Range Test' (DMR), (Steel and Torrie, 1992) and Tukey's HSD test were applied for comparison of mean values (Snedecor & Cochran. 1991). MSTATC, MICROSTAT and SPSS Packages of the computer software were used to analyze the data.

Results

The proximate nutrient analysis of feed, feces, and estimation of chromic oxide are shown in Table –3.

Apparent digestibility for test ingredients

Apparent digestibility co efficient values of DM, CP, lipids and GE of the test ingredients (Fish meal,

Sunflower meal and Rice polishing) are summarized in Table 4. Apparent nutrient digestibility coefficient of DM was highest (67.77% ± 6.81) for fish meal followed by sunflower meal (38.18% + 0.63) and rice polishing (21.63% ± 20.46). The analysis of variance of DM showed that the apparent DM digestibility of all the three tests ingredients were statistically non-significant (P >0.05)

Apparent CP digestibility was highest (88.84% ± 4.90) for the fish meal and was followed by sunflower meal (71.46% ± 1.54) and rice polishing (70.22% ± 8.62). The results of the statistical analysis of CP affirmed that the apparent CP digestibility of all the three test ingredients were also statistically non-significant (P > 0.05).

For crude fat apparent digestibility was highest (82.37% ± 6.26) for sunflower meal and next highest values in the descending order were (80.36% ± 3.07) for fish meal and (38.38% ± 1.51) for rice polishing. The analysis of variance of crude fat digestibility showed that the apparent crude fat digestibility of test ingredients were statistically significant (P < 0.05).

For any two means to be significantly different from each other, their difference must be more than the critical value (Rp). In case of crude fat, the values obtained from DMR test shows that none of the two means were significantly different from each other. Tukey's test which yielded better results showed that there, existed a significant difference between rice polishing and fish meal.

The apparent digestibility for GE was highest (93.64% ± 9.34) for fish meal and this was followed by the rice polishing (77.47% ± 10.02) and sunflower meal (67.29 % ± 3.50). The statistical analysis concluded that the apparent gross energy digestibility percentage of the test ingredients were statistically non-significant (P > 0.05).

Table 2: Proximate chemical analysis (%) of reference and test diets

Chemical composition	Reference diet	Test diet I	Test diet II	Test diet III
DM (%)	94.72	94.32	96.24	95.34
CP (%)	28.43	31.65	26.26	21.4
Crude fat (%)	36.51	31.83	18.72	18.6
Gross energy (Kcal/100g)	29.45	33.58	36.66	36.65

Table 3: Percentage of crude protein. Crude Fat, Gross Energy and Marker Contents in Diets and Faeces (Mean ± S.E, n = 3 DM basis)

Component	Reference	Test diet I	Test diet II	Test diet III
Diets				
CP (%)	28.43 ± 0.06	31.65 ± 0.04	26.26 ± 0.04	21.40 ± 0.04
Fat (%)	36.51 ± 0.18	31.84 ± 0.04	18.72 ± 0.20	18.60 ± 0.13
Gross energy (Kcal/100g)	29.45 ± 0.08	33.58 ± 0.10	36.66 ± 0.03	36.65 ± 0.20
Chromic oxide (%)	1.06 ± 0.02	1.06 ± 0.01	1.09 ± 0.01	1.07 ± 00.01
Faeces:				
CP (%)	6.93 ± 0.96	8.72 ± 0.66	9.12 ± 0.96	7.29 ± 0.36
Fat (%)	7.28 ± 2.48	8.11 ± 2.24	4.86 ± 1.08	7.99 ± 0.63
Gross energy (Kcal/100g)	18.66 ± 0.31	17.11 ± 1.04	21.81 ± 2.03	18.31 ± 0.49
Chromic oxide (%)	3.47 ± 0.88	3.36 ± 0.36	2.66 ± 0.01	2.44 ± 0.61

Table 4: Apparent Nutrient digestibility Coefficient (%) of test Ingredients (Mean ± SE, n= 3) using Chromic Oxide as Marker

Diet	DM	C. Protein	C. Fat	Energy
Fish meal	67.77 ± 6.81	88.84 ± 4.90	80.36 ± 3.07	93.64 ± 9.34
Sunflower	38.18 ± 0.63	71.46 ± 1.54	82.37 ± 6.26	67.29 ± 3.50
Rice polishing	21.63± 20.46	70.22 ± 8.62	38.38 ± 1.51	77.47 ± 10.02

Discussion

The apparent digestibility of nutrients in the test ingredients was higher in animal ingredient (fish meal) than plant ingredients (sunflower meal and rice polishing). The apparent digestibility of DM was comparatively lower for sunflower meal and rice polishing. By comparison, the DM digestibility of fish meal was higher but statistically there was no significant difference in DM digestibility among plant and animal ingredients as well as between plant ingredients. This result is partially in agreement with Burel *et al.* (2000). They observed that no significant difference in the digestibility of DM among the plant ingredients, due likely to the low statistical power of the experiment. The results of low apparent DM digestibility of current study are also in line with Hajen *et al.* (1993). They reported low DM digestibility in plant ingredient with high carbohydrate contents.

The low digestibility of DM for plant ingredients in the present study may be due to higher carbohydrate contents. Several other studies reported low DM digestibility coefficients in plant protein with high carbohydrate contents (Allan *et al.* 2000 and Laining *et al.* 2003). However, Sugiura *et al.* (1998) suggested that fish cannot utilize non-protein component from plant material effectively because of the presence of starch and fibers.

The apparent digestibility for CP was higher in animal ingredient as compared to plant ingredients. Of the ingredients tested in this study, fish meal (animal ingredient) was most digestible with an apparent protein digestibility (APD) of 88.84% $\pm$ 4.90 and for sunflower meal and rice polishings (plant ingredient), apparent protein digestibility values were lower.

The APD of fish meal is nearly the same reported by Hossain and Jauncey (1989). They observed APD value of 88.9% for fish meal in carp. Similar value of fish meal digestibility for carp was also reported by Kim (1974). According to (Annonymous, 1997) carp can digest up to 95% of protein in fish meal. However, the value can decrease to 80-85% depending on the origin and processing of fish meal concerned (Ogino and Chen, 1973). The higher apparent digestibility for fish meal might be the contribution of amino acid profile which are well balanced in fish meal than in sunflower meal and rice polishing. Allan *et al.* (2000) observe that essential amino acid contents profile availability in fish meal were superior. These findings of the study concurred with the results of Jalal *et al.* (2000), where digestion coefficient of protein in fish meal (95.13%) was higher than wheat meal (84.70%) and superiority of fish meal was due to their balanced amino acid profile. The low APD in plant ingredients might be due to higher contents of carbohydrates. Falge *et al.* (1978) and McCartney (1971) showed that trout amylase activity was affected by the type and amount of carbohydrate in the diet and that increasing carbohydrate load in the diet generally resulted in a decrease in enzyme activity. Wee (1992) concluded that undigested carbohydrate passed rapidly out of the gut taking some undigested protein with it, thus affecting protein digestibility.

The current study showed that the crude fat in sunflower meal (82.37% $\pm$ 6.26) and fish meal (80.36% $\pm$ 3.07) was well digested by *Labeo rohita*. Where as the digestibility for rice polishing (38.38% $\pm$ 1.51) was poor. The values of sunflower meal and fish meal did not show any significant difference ($P > 0.05$). The crude fat digestibility values are lower than the values (85 to 95%) reported by (Annonymous, 1993).

By comparison, the digestibly of crude fat in fish meal was slightly lower to the value (81.80%) reported by Jalal *et al.* (2000). However, fat digestibility of present study was higher than the value (68%) as reported by Gaylord and Gatlin (1996). They concluded that some of the difference in lipid digestibility values for red drum compared to other species might be attributable to differences in techniques used to extract lipid.

The apparent energy digestibility (AED) of fish meal was higher (93.64 $\pm$ 9.4) than plant ingredients i.e. rice polishing (77.47 $\pm$ 10.02) and sunflower meal (67.29 $\pm$ 3.50). The AED for fish meal in the present study was also higher than reported by some other researchers (74%, Windell *et al.* 1978, 91%, Cho *et al.* 1982; 91.5%, Smith *et al.* 1980) for rainbow trout, (83%, Law 1986) for grass carp and (73.5%, Kirchgessner *et al.* 1986) for common carp. These lower AED values of fish meal, could possibly be due to higher crude fiber content which accelerate the rate of passage of digesta through the intestinal tract thus reducing the digestibility of energy and protein (Hilton *et al*. 1983).

The AED of plant ingredients (sunflower and rice polishing) in current study was comparatively lower than animal ingredient (fish meal). The lower AED of plant ingredients could be attributed to their higher carbohydrate contents and poor digestibility by carnivorous fish (Lupatsch *et al*. 1997). Similar confirmation was reported by Storebakken *et al* (1998). They concluded that increased dietary carbohydrate (10-20%) reduced DM, energy and fat digestibility but had little effect on protein digestibility for rainbow trout. Generally, freshwater and warm water fish appear to digest carbohydrate more efficiently than carnivorous and coldwater fish. The factor affecting AED could possibly be due to more leaching of nutrient in animal ingredient. This

is agreed with the findings of Watanabe *et al*. (1996), who concluded that leaching accounted for an increase in digestible energy coefficient, for rainbow trout.

In conclusion, *Labeo rohita* were able to digest energy and nutrients of the animal source (fish meal) more efficiently than plant ingredients (sunflower meal and rice polishing), though the percentage of nutrient digestibility of sunflower meal and rice polishing was comparatively less but all the digestibility values were somewhat near to standard digestible values of carps (Annonymous, 1993). The variations among replicates of each test ingredients might be due to experimental error like leaching of nutrients need further confirmation. However, the data established in this study provided the basis for inclusion of fish meal as well as sunflower meal and rice polishing for the formulation of diet for *Labeo rohita*.

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